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Review article

The role of matrix metalloproteinases in infectious corneal ulcers

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ABSTRACT

During infectious keratitis, the production of collagenolytic and inflammatory substances, along with increased corneal matrix metalloproteinase (MMP) activity, induces the degradation of corneal collagen and may cause postkeratitis complications, such as opacity, thinning, and corneal perforation. MMPs, especially MMP-2 and MMP-9, are overexpressed in infectious keratitis and sustained over time by inflammatory and nonmicrobial mechanisms. The high MMP levels are correlated with excessive corneal destruction in bacterial, herpetic, fungal, and acanthamoeba infections. Nonspecific treatments, such as tetracyclines, particularly doxycycline, or corticosteroids, are used as adjuvants to antimicrobials to alleviate the disproportionate degradation and inflammation of the corneal layers caused by corneal MMPs and decrease the recruitment and infiltration of inflammatory cells. Treatments showing inhibition of specific MMPs (Galardin, ZHAWOC7726), interfering with pro-MMP activation (EDTA, ascorbic acid), or showing anticytokine effect (epigallocatechin-2-gallate, TRAM-34) have been reported. Other treatments show a direct action over corneal collagen structure such as corneal cross-linking or have been associated with reduction of MMP levels such as amniotic membrane grafting. Although the use of these drugs has been shown in studies to be effective in controlling inflammation, especially in experimental ones, robust studies are still needed based on

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randomized and randomized clinical trials to demonstrate their potential effect as adjuvants in the management of infectious keratitis.

1. Introduction

The extracellular matrix (ECM) of the cornea is a complex and organized meshwork comprised of cells, especially keratinocytes, collagen, proteoglycans, hyaluronic acid, elastin, laminin, fibronectin, and other components, such as matrix metalloproteinases (MMPs), immune mediators, and growth factors that compose and maintain the corneal homeostasis and its characteristic transparency.[81,109]

In a healthy cornea, ECM and stromal collagen remodeling is mediated by the MMPs secreted by keratocytes.[34,103] MMPs are zinc-dependent endopeptidases with a main proteolytic role in degrading and remodeling ECM components.[18,78] Based on their chemical substrate and function, MMPs are divided into membrane-type-MMPs, collagenases, gelatinases, stromelysins, and matrilysins.[27] The action of MMPs is controlled by a complex molecular interplay. Tissue inhibitors of MMPs (TIMPs) are the main endogenous regulators of MMPs,[4] with an important function in uptaking, activation, and removal of MMPs, as well as influencing cell phenotype and production of cytokines, chemokines, and growth factors.[4,18,100] The impairment of the balance between MMPs/TIMPs is implicated in multiples diseases, such as diabetes, hypertension, renal diseases, neurodegenerative diseases, wound healing, cancer, and inflammatory disorders.[18]

Likewise, the disruption of the corneal surface homeostasis because of MMPs/TIMPs imbalance has an important role in diverse conditions, including infectious keratitis and its progression.[17] Microbial keratitis causes an excessive release of proteolytic enzymes, especially MMPs, along with the transformation of quiescent keratocytes in activated fibroblasts, as well as indirect recruitment of inflammatory cells, thus preventing the healing of the ulcer and causing corneal melting by collagen destruction, neovascularization, corneal scar formation, and even corneal perforation.[34,73] Although certain MMP inhibitors have been used as adjuvants in non-infectious ulcers, and their positive effect on ulcer healing has already been advocated in moderate to severe ocular chemical injuries and sterile corneal ulceration, among others,[84,79] their use is still controversial. In fact, the overall knowledge regarding their use in the management of corneal infections is currently scarce.

As far as we know, there are no ongoing clinical trials attempting to clarify the role that MMPs and TIMPs may have in the pathophysiology of infectious corneal ulcers, nor the use of MMP inhibitors as adjuvants in the therapeutic management of microbial keratitis.

We focus on the participation of MMP and TIMP infectious keratitis, their relationships, and their role in stromal corneal destruction, remodeling, neovascularization, and the different strategies to control corneal degradation.

2. Metalloproteinases and the ocular surface

MMPs and TIMPs are present in the ECM of the different structures of the eye.[17] On the ocular surface, MMPs help to maintain the normal corneal structure and play an important role in the destruction and remodeling of corneal components.[27]

MMPs are produced as pro-MMPs that depend on zinc for their catabolic activation in the ECM.[27] The production of MMPs is regulated by various activators and inhibitors. At transcriptional levels, the expression of MMPs is upregulated by mRNA expression, hormones such as estrogen and progesterone, inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), and growth factors including transforming growth factor-β and β1 (TGF-β, TGF-β1) and endothelial growth factor (VEGF), cell-to-cell contact, and ECM interactions.[27,76] For example, the overexpression of VEGF increases MMP-2 concentrations in rats. In contrast, TGF-β1 may downregulate MMPs.[38] In addition to the gene transcription level, MPPs are regulated at posttranslational levels with the modifications of proteins by mRNA stability, epigenetic regulation, and the action of enzyme precursors with endogenous and exogenous activation.[59]

The activation of pro-MMPs requires the disruption of the cysteine switch through zinc in the catalytic site. Proteinases including plasmin, trypsin, other MMPs, reactive oxygen, and hypochlorous acid produced by leukocytes can facilitate this disruption and the MMP activation.[76] Once activated, MMPs are regulated by unspecific protease inhibitors, such as α2-macroglobulin and α1-antiprotease, and by specific inhibitors, the TIMPs. Endogenous TIMPs, namely, TIMP-1, − 2, −3, and -4, blockage the activity of all MMPs in a reversible form.[27] Increased MMPs or decreased TIMPs levels can lead to MMP/TIMP imbalance, resulting in pathological conditions.[27]

Under physiological conditions, the balance between MMPs and TIMPs is essential to maintain corneal homeostasis. Excessive levels of MMPs cause a disbalance of this ratio that can be tested in the tear film and has been linked to dry eye syndrome, corneal ulceration or erosion, and other inflammatory conditions.[37,48] MMP-2 (gelatinase or collagenase A) comes from stromal fibroblasts and macrophages. Its function in a healthy cornea consists of breaking down collagen types IV, V, VII, gelatins, and fibronectin.[32] In pathologic conditions like after an injury, however, MPPs, including MMP-2, show abnormally elevated levels.[17] MMP-9 is a collagenase B originating from corneal epithelial cells, macrophages, and neutrophils with similar substrates to MMP-2[78] but with different patterns of action. The long-time persistence of MMP-2 after an injury suggests that it is involved in collagen remodeling and stromal reparation, while MMP-9 might participate in the degradation of epithelial basement membrane before corneal ulceration.[32] MMP-1 (collagenase A) breaks

down collagen types I, II, and III and is produced by stromal fibroblasts and keratocytes, while MMP-3 (stromelysin) breaks proteoglycans, fibronectin, laminin, gelatin, and types III, IV, and V collagen.[27] MMP-8, a neutrophil collagenase, also digests types I, II, and III collagens, but it is only produced by polymorphonuclear polymorphonuclear neutrophils (PMNs). In certain infectious and inflammatory conditions, MMP-2, MM-8, MMP-9, and other MMPs are also secreted by inflammatory cells including fibroblasts (that originate from activated keratocytes), endothelial cells, macrophages, neutrophils, and lymphocytes.[27,32,49] Moreover, TIMP-1 TIMP-2, and TIMP-3 are normally present in the cornea and can be produced by the same cells that express MMPs.[51]

3. Metalloproteinases and microbial keratitis

3.1. *Metalloproteinases and bacterial keratitis*

Bacterial keratitis implies an intense interaction with corneal ECM that determines the urgency, progression, resolution, and implications of the infection.[97] The role of MMPs in bacterial keratitis has been widely studied. Collagen degradation is mediated by proteases and enzymes released by the infecting bacteria and the inflammatory cascade precipitated by infection and corneal fibroblasts.[34] (Fig. 1).

Corneal epithelial cells contain pattern-recognition receptors that can generate innate immune responses against microbial factors known as pathogen-associated molecular patterns.[97] The pathogen-associated molecular patterns released are different depending on the bacterial type. Gram-negative bacteria such as *Pseudomonas aeruginosa*, the most common bacterium causing infectious keratitis and frequently associated with severe corneal melting,[95] release lipopolysaccharide, a type of pathogen-associated molecular patterns. Toll-like receptors are part of the innate immune system, and they recognize a wide range of patterns from many infectious agents. Quiescent corneal keratocytes differentiated into corneal fibroblasts and myofibroblasts can detect lipopolysaccharide and other virulence factors through toll-like receptors, activating innate immune response by means of inducing the secretion of MMPs, TGF-β1, and chemokine interleukins IL-1 and IL-8.[34] These activated fibroblasts induce, in turn, urokinase-type plasminogen

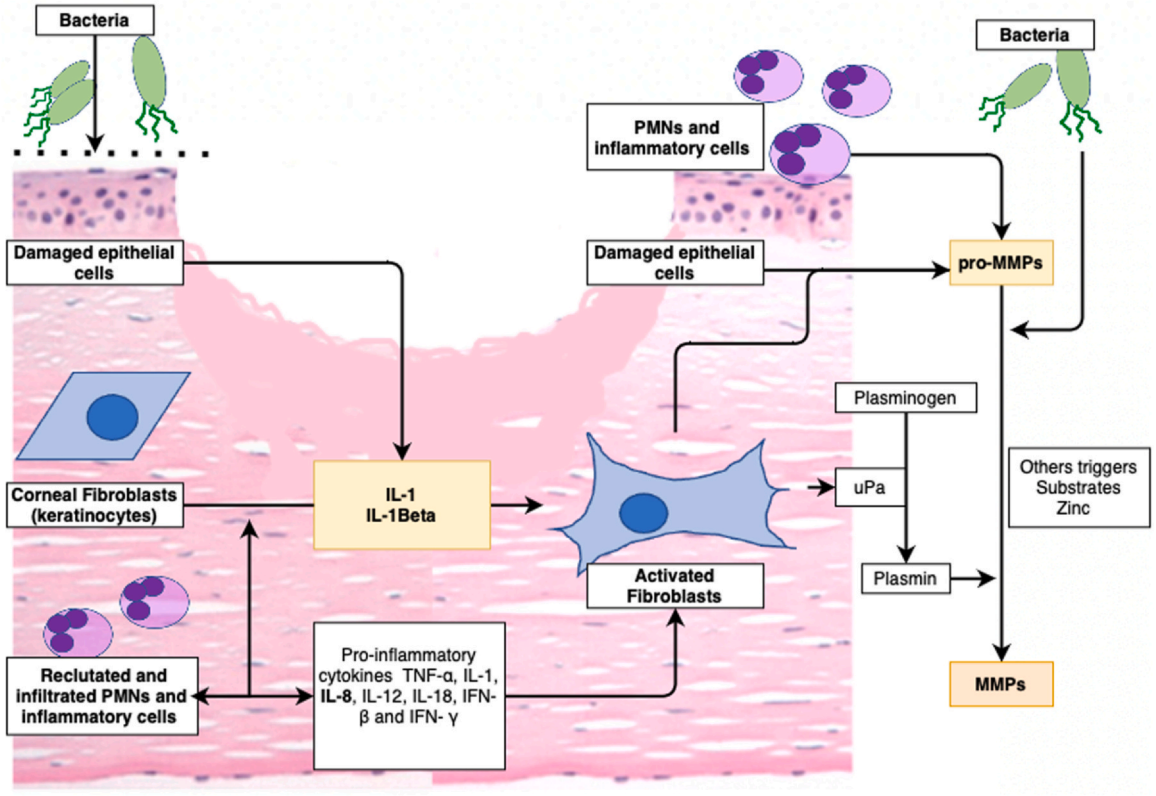

Fig. 1 – Role of keratinocytes, inflammatory cells, and epithelial corneal cells in the production of metalloproteinases (MMPs) in bacterial keratitis. In the left area of the graphic, the depiction shows how bacteria activate epithelial corneal cells, keratinocytes, and inflammatory cells, namely, polymorphonuclear neutrophils (PMNs). This interaction induces the secretion of interleukins and cytokines, initiating an inflammatory cascade that causes the activation of corneal fibroblasts. In the right area of the graphic, it is shown how diverse types of pro-MMPs are produced by epithelial corneal cells, activated fibroblasts, and PMNs. The transformation in the activated form of MMPs requires the interaction of activated fibroblasts with the urokinase-type plasminogen activator (uPA)-plasmin-MMP via the direct effect of specific bacterial products or other triggers in a positive microenvironment with enough substrates.

activator (uPa). The uPa transforms the plasminogen into plasmin, which mediates the conversion of inactive pro-MMPs into their active forms, thus releasing their proteolytic actions.[93] The uPA-plasmin-MMP via plays a central role in the disproportionate collagen degradation during corneal ulceration, with MMP-1 and MMP-9 as the main mediators.[12,93] Moreover, fibroblasts recruit inflammatory cells that release proinflammatory cytokines, including TNF-α, IL-1, IL-8, IL-12, IL-18, IFN-β, and IFN-γ.[97]

To promote collagen destruction, *Pseudomonas aeruginosa* produces proteases, including MMPs, serine proteases, and other collagen-degrading enzymes, and helps to activate pro-MPPs secreted by fibroblasts.[97,34] Other bacteria, such as *Streptococcus pneumoniae*, release virulence factors, including a polysaccharide, pneumolysin, neuraminidases, and zinc MMPs.[10] In addition, other bacteria release IL-1β that induces the expression of IL-8, a potent neutrophil-attracting molecule, thus enabling the prolongation of the MMP cascade.[12,75]

To facilitate bacterial clearance, PMNs generate an innate immune reaction in the infection site and the secretion of IL-1 and IL-1β, and stimulate corneal fibroblasts into the production of MMPs. The interaction between PMNs and corneal fibroblasts promotes the further infiltration of inflammatory cells. Thus, activated corneal fibroblasts are responsible for collagen-degrading MMPs secretion and the sustaining of corneal ulcer physiopathology.[86,102]

Likewise, activated corneal fibroblasts stimulate the upregulation of MMP-1, MMP-2, and MMP-9 and the downregulation of TIMP-1.[47,66,102] Numerous studies support a correlation between the high levels of MMP-9 in acute *Pseudomonas aeruginosa* keratitis and the severity of the keratitis.[46,64,102] In mice, the neutralization of MMP-9 produced by *Pseudomonas aeruginosa* reduced Langerhans cells and PMN count, reducing the severity of the corneal ulcer.[64] MMP-9 has also been proposed as an indicator of inflammatory eye disease and ocular surface disease and suggested as a therapeutic target.[50]

Therefore, according to the evidence, the intervention in the early stage of the ulcerative keratitis, inhibiting the subsequent cascade of events regulated by corneal MMPs, constitutes an initial opportunity for therapeutic intervention.

3.2. *Metalloproteinases and virus*

Viral infections share with bacterial keratitis some events related to collagen destruction. In fact, *Herpes simplex* virus (HSV) keratitis generates an upregulation of MMP-9.[56] The infection of human epithelial cells by HSV produces an upregulation of MMP-9 and TNF-α in a dose-dependent manner. In fact, TNF–α concentration is positively correlated with MMP-2 and MMP-9 levels.[106] The upregulation of MMP-9 seems to have specific timing, with MMP-9 levels increasing 2 days after inoculation of HSV and decreasing at 7 days after infection. The decrease in PMN infiltrates areas, and TNF-α levels are linked with the downregulation of MMP-9.[105]

The inhibition of inflammatory cells such as macrophages[42] and PMNs[56] in HSV keratitis in mouse models decreases the inflammatory cell infiltration and MMP-9 levels. The ratio of MMPs to TIMPs may be important for the course of necrotizing HSV keratitis since TIMPs might participate in the repair process.[105] In stromal ulceration, the neutrophil infiltration might provide a source of MMP-8 collagenases. Likewise, IL-1β levels seem to cause an imbalance in the level of MMPs and TIMPs, and have also been linked with the severity of HSV corneal infection.[102]

Furthermore, MMP-2, MMP-7, and MMP-9 have an interesting role in corneal repairing, remodeling, and angiogenesis. MMP-2 concentration increases during corneal neovascularization in the areas of new vessel formation,[54] just like MMP-14, which stimulates migration and provides guidance for vascular endothelial cells during neovascularization.[20,91] PMN and macrophage upregulation caused by MMP-9 activity stimulates neovascularization. Meanwhile, the inhibition of MMP-9 seems to be associated with a reduction of corneal angiogenesis in HVS keratitis.[56] Therefore, the incorporation of these inhibitors in clinical practice has a therapeutic potential for improving the final corneal status. However, this should be still demonstrated in randomized, blinded clinical trials.

3.3. *Metalloproteinases and fungi*

TIMPs and MMPs, especially MMP-9, play a relevant role in the pathophysiology of fungal keratitis.[25,67] Excised corneal buttons with previous fungal keratitis demonstrated a significant increase in MMP-9 activity, inflammatory cytokines, and PMN count in a study.[25] In an experimental model of *Candida albicans* keratitis, it was found that transcriptional levels of MMP-8, MMP-9, MMP-13, and TIMP-1 were increased in the early stage and were linked to the progression to a more severe stage of keratitis.[25,108] Other studies have confirmed the expression of MMP-8 and MMP-9 derived from leukocytes and PMNs in *Fusarium solani* keratitis. The expression of MMP-2, MMP-3, MMP-7, and MMP-13 was also found to be augmented, and TIMP-1 was found to be upregulated, whereas TIMP-2, -3, and -4 concentrations did not show significant changes.[58] Therefore, the MMP/TIMP ratio may be important to determine the course of fungal keratitis.[58] Furthermore, microbial collagenases have been also found to be produced by fungi.[29]

3.4. *Metalloproteinases and acanthamoeba*

Acanthamoeba trophozoites first adhere to epithelial cells and then produce extracellular proteases that promote the destruction of corneal tissues[63,68] and collagenolytic enzymes that cause severe stromal necrolysis. These enzymes were found in experimental studies injecting Acanthamoeba into the cornea of rats.[6] On the other hand, the study of other proteins secreted by Acanthamoeba in *in vitro* conditions, such as the mannose-induced Acanthamoeba cytopathic protein (MIP-133), showed that it does not cross-react with MMP-1 and MMP-9 in epithelial and stromal cells. MIP-133 regulated MMP-2 and MMP-3 expressions in a different manner depending on the cell type,[1] a fact that illustrates the different targets of Acanthamoeba enzymes.

4. The role of metalloproteinases in microbial keratitis treatment

Antimicrobials are the treatment of election in infectious keratitis; however, the current therapies are focused exclusively on the antibiotic effects, neglecting the stromal degradation cascade triggered by activated fibroblasts and the disproportionate activity of MMP that occurs after the elimination of the microorganism. Acute and chronic inflammatory changes in corneal stroma cause structural alterations and may induce long-term vision loss. In fact, the very antimicrobial therapy can promote the release of bacterial factors and the initiation of the collagenolytic cascade, which, in the first instance, may elicit corneal melting, and corneal fibrosis and angiogenesis posteriorly.[45]

There are some potential strategies to control corneal melting through MMP inhibitors (Fig. 2). The direct suppression of MMPs has been explored with inhibitors such as ZHAWOC7726, a synthetic TIMP peptidomimetic protein. Using native inhibitors of MMPs, such as TIMPs, permit high inhibitory ability and differences in specificity.[57] The synthetic inhibition of MMPs with molecules such as ZHAWOC7726 has been shown promising in preclinical studies for the treatment of a variety of related diseases, including rheumatoid arthritis, inflammatory processes, and cancer. TIMP-1 binds to pro-MMP-9 and prevents the conversion of MMP-9 into an active enzyme. ZHAWOC7726 downregulated MMPs-9 expression and its proinflammatory action in *ex vivo* models and reduced ulcer progression, corneal perforation, and scar formation in infectious *Pseudomonas aeruginosa* keratitis in a porcine model.[73]

Galardin (GM6001 or Ilomastat) is another small-molecule inhibitor of MMP, with a broad spectrum of action against MMP-1, -2, -3, -8, and -9, which has been shown to have a significant effect in inhibiting MMP-2 and MMP-9 activity in *in vitro* condition. Topical treatment with galardin seemed to prevent and delay the onset of corneal melting and perforation in experimental models of keratitis by *Pseudomonas aeruginosa*,[9,16,41] and the topical treatment with ilomastat

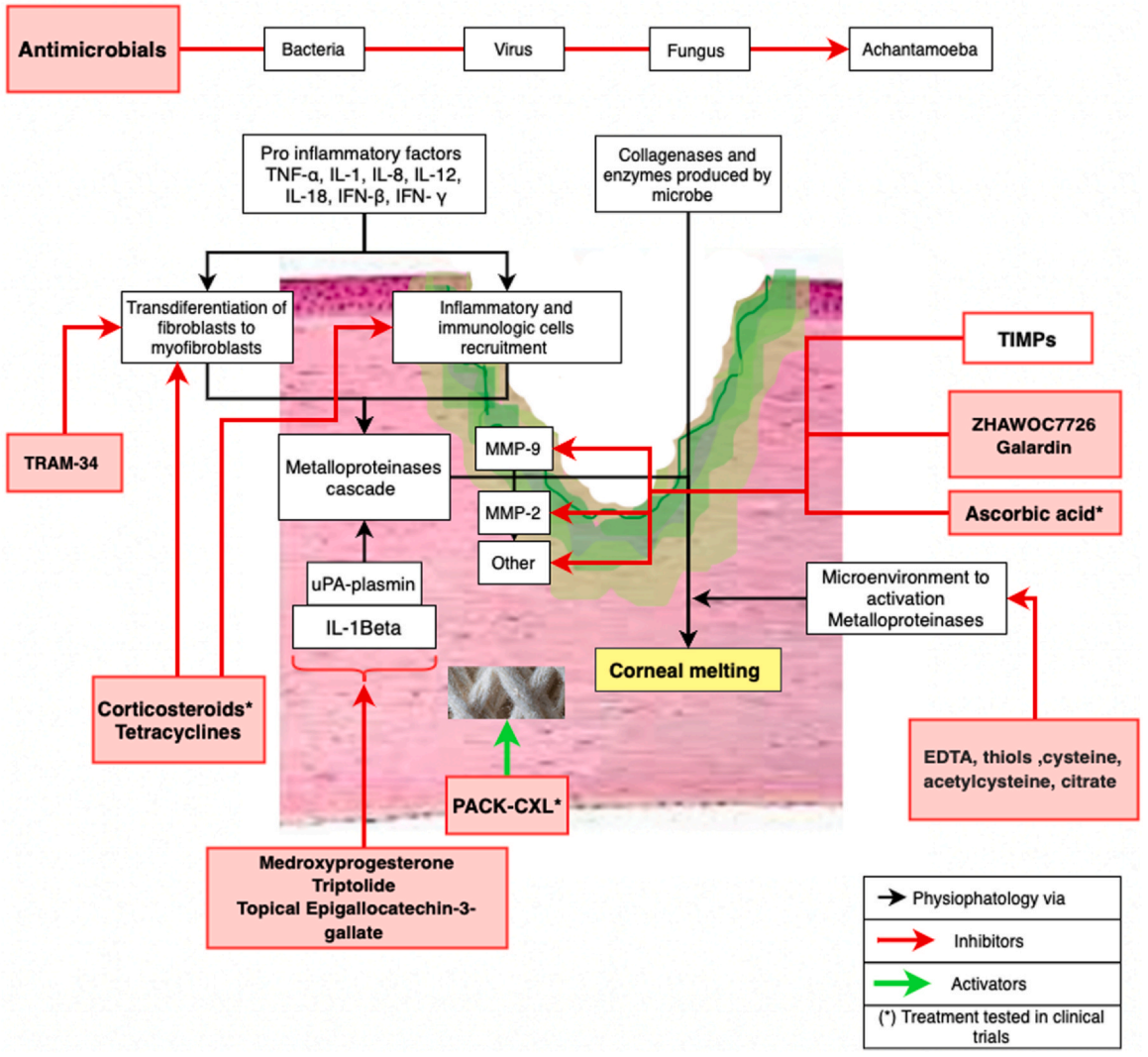

Fig. 2 – Diagram of antimetalloproteinases treatment and its blocker points in infectious keratitis. uPA, urokinase-type plasminogen activator. MMP, matrix metalloproteinase. TIMP, tissue inhibitor of MMP.

presented a therapeutic concentration in the sclera, conjunctiva, and aqueous humor in a rabbit model.[71] Galardin has been studied in preclinical models and clinical trials for the treatment of various conditions related to elevated MMP activity involving cancer, cardiovascular disease, and periodontitis. In preclinical studies, galardin has been associated with side effects, such as inhibition of normal wound healing, altered tumor progression, and increased susceptibility to bacterial infections[7,69]; however, the evaluation of these side effects in the human ocular surface has not been studied.

Although the peptides galardin and ZHAWOC7726 have shown promise in preclinical studies, they have not yet been approved for use in humans.

The inhibition of selected MMPs could be used as adjunctive therapy in bacterial keratitis. The inhibition of MMP-13 activity with subconjunctival and topical inhibitor treatment (MMP13i) may contribute to decreasing the basement membrane degradation by *Pseudomonas aeruginosa* and its severity in a mice model.[36]

Likewise, the selective inhibition of MMP-2 and MMP-9 by the small-molecule SB-3CT has been shown to reduce corneal inflammatory lymphangiogenesis in a murine model.[28]

The application of peptides as drug molecules *in vivo* and clinical conditions has been limited because of intrinsic high *in vivo* clearance, low molecular stability, and low membrane permeability.[35] Likewise, due to the structural homology shared by MMPs, most developed inhibitors have a broad spectrum of inhibition and affect the bilateral role of MMPs in pathological situations, such as cancer, angiogenesis, invasion, or viral and bacterial infection, in which MMPs present a procarcinogenic and anticarcinogenic effect or prosusceptibility and antisusceptibility to infection. The biochemical studies promote that the designing of MMP inhibitors must be highly specific and selective to limit side effects and ideally administered as topical and a short-term treatment.[57]

In addition, antibody-based inhibitors of MMPs displayed preferential binding. Being needed verifies that the developed drug presents the preference for human MMPs.[57]

Collagenases are also inhibited by metal-binding agents, such as ethylenediaminetetraacetic (EDTA), thiols,[15,16] cysteine, acetylcysteine, citrate,[80] and human serum antiprotease α2-macroglobulin, due to the zinc requirement of MMPs and other proteolytic enzymes to be activated.[11] Some formulations have been used for this chelating and anticollagenase effect as adjuvants for conventional therapy. For example, the combined use of a gel of 0.02% chlorhexidine digluconate and chelating agent EDTA achieved a drastic reduction of trophozoite and cyst survival of Acanthamoeba keratitis in *in vitro* studies.[26,8] EDTA could be useful in the treatment of gram-negative bacterial[61] and fungal[60] keratitis due to its chelating properties and direct effect on different structures of microbes. These agents, however, despite high affinity and favorable results *in vitro* and some experimental animal studies, present a poorly bioavailability *in vivo* due to their unspecific inhibition of MMPs and other unrelated proteins that may be affected by the metal chelation and consequently have not elicited clinically significant responses in patients.[57]

Inhibiting corneal fibroblasts and inflammatory cells, the source of MMP cascade, is other alternative to reduce corneal melting.[34] Tetracyclines present both broad-spectrum antibiotic and nonantimicrobial properties, including tissue inhibition of MMPs, cytokines, and growth factors.[31] Tetracyclines have the ability to chelate calcium and zinc ions necessary for the activation of MMPs and show anticollagenolytic action and activity against neutrophils, immunomodulation, cell proliferation, and angiogenesis.[13,31] Within this antibiotic group, doxycycline has been shown to inhibit JNK, ERK, and p38MAPK signaling pathways and to reduce MMP-9 activity, decrease keratocyte MMP-9 expression, and reduce TNF-α and TGF-β transcription.[52,77,89,90] A reduction of corneal melting was reported with systemic doxycycline use in chemical and sterile ulcer cases.[84,79,31] In the infectious ulcer area, the use of oral doxycycline 100 mg twice a day during a period of four weeks may help to stabilize corneal breakdown as an adjunctive therapy in the management of *Pseudomonas eruginosa*,[65] Acanthamoeba,[82] Nocardia,[96] and *Candida albicans*[14] corneal melting. However, to the best of our knowledge, no randomized clinical trials have been conducted regarding the efficacy of doxycycline, nor other tetracyclines, as adjuvants for the treatment of infectious ulcers. Regarding lymphangiogenesis and angiogenesis, topical treatment with doxycycline inhibited the corneal lymphangiogenesis dramatically through the suppression of VEGF-C signaling, macrophage function, and MMPs activity, especially of MMP-9.[31,40,107] The development of the third generation of tetracyclines such as tigecycline, omadacycline, and eravacycline may have anti-inflammatory properties and anti-MMP activity *in vitro*, specifically against MMP-2 and MMP-9. However, it is necessary for studies to value the clinical effectiveness in treating corneal ulcers or other ocular surface diseases.[87] By contrast, topical administration of other antibiotics, such as fluoroquinolones, may induce the expression of MMP-1, MMP-2, MMP-8, and MMP-9 in debrided corneal epithelium, thus delaying corneal wound healing.[88,85]

Comparing the topical effect of doxycycline to dexamethasone in a model of corneal alkali burns in mice, dexamethasone was associated with the lowest corneal opacity scores and a greater effect decreasing MMP levels, neutrophil infiltration, inflammatory cytokines, TIMP-1 expression, and a significant increase in MMP-8. On the other hand, topical doxycycline showed faster wound healing and fewer corneal opacities than placebo treatment.[13] Steroids are used in corneal ulcers and controlled keratitis infections to reduce the inflammatory cascade, corneal neovascularization, and fibrosis. Steroids may improve corneal haze, scar, and fibrosis, attenuating cell-mediated collagen degradation and the infiltration of inflammatory cells.[5] Several clinical trials regarding the benefit of corticosteroid treatment in infectious ulcers have been performed, but no specific conclusions have been reached.[5] Actually, the SCUT trial could not find an overall difference at 3 months in best-corrected visual acuity. Only using a regression model, it was found that corticosteroid use, initiated after 48 hours of the antibiotic treatment, was associated with a mean improvement of one line in best-corrected visual acuity at 12 months among patients with ulcers not caused by Nocardia.[74,92] Hopefully, the SCUT-II study will provide new evidence in this regard.[83] Topical corticosteroids are also used as adjuvant therapy to VHS corneal stromal disease. Topical corticosteroid treatment in VHS reduces the persistence and progression of stromal inflammation and contributes to faster resolution of the

infection without detrimental effects in visual outcomes at 6 months in comparison with noncorticoid groups.[5101] Similarly, corticosteroid treatment has been used to control the inflammation induced by *Acanthamoeba* after anti-acanthamoeba treatment has been initiated.[19]

Photoactivated chromophore for corneal cross-linking (PACK-CXL) associated with corticosteroids as adjunctive treatments in bacterial keratitis has provided evidence of their efficacy in reducing inflammatory cells and bacterial pathogens.[83] Several studies and meta-analyses support the benefit of PACK-CXL in bacterial keratitis[62] since PACK-CXL may increase the resistance of keratolysis by inhibiting collagenase A, MMP-2, in *in vitro* models;[3] however, the relation between ultraviolet B radiation and other MMPs such as MMP-9 is controversial.[48] Moreover, there are still some concerns regarding the use of PACK-CXL in nonbacterial infectious keratitis.[39] Again, more evidence supported by randomized clinical trials is necessary.

The inhibition of other molecules, such as proinflammatory cytokines, may also be useful in the prevention of corneal melting caused by stimulation of MMPs activity. TNF-α is an inductor MMPs activity. In corneal melting refractory to treatments such as topical and systemic steroids, steroid-sparing agents and amniotic membrane transplantation showed a response with the infusion of infliximab (a TNF-α inhibitor), which downregulated the expression of MMPs associated with collagen breakdown.[98] Another possible therapeutic target for corneal ulcers is the uPA-plasmin system. Medroxyprogesterone inhibits IL-1β-induced collagen degradation and the upregulation of uPA expression by corneal fibroblasts, MMPs expression and activation, and TIMPs expression.[110,111] Medroxyprogesterone has not been tested in infectious keratitis, however. Similar to medroxyprogesterone, triptolide is able to inhibit IL-1 and lipopolysaccharide-induced chemokines[53,110] and could be useful as a potential treatment for corneal ulcerations, especially those of viral origin. Topical epigallocatechin-3-gallate suppressed the IL-1β-secretion and attenuated the formation of corneal neovascularization via inhibition of VEGF production and reduction of MMP-9 levels in chemical ulcers in mice corneas.[24,30,70]

The inhibition of secretion inflammatory factors IL1-β and IL-10 by atractylenolide I[22] and thymol,[99] through the suppression MyD88/NF-κB pathway, combined with natamycin get increase corneal transparency in an experimental model of mice infected by *Aspergillus fumigatus*. Likewise, in *A. fumigatus* keratitis model, the infiltration of nerolidol inhibits the production of LOX-1/IL1-β signaling,[104] and the use of astaxanthin inhibits IL1-β and TNF-α[44] protecting against the inflammation damage of the cornea. The selective suppression of interleukins, especially in fungal keratitis, may be a promising treatment in the corneal clearance in light of experimental murine studies.

Regarding corneal neovascularization and corneal opacities prevention, ascorbic acid, via anti-VEGF and anti-MMP mechanisms, inhibits VEGF and MMP-9 production, and its topical application decreased neovascularization in a rabbit corneal model.[55] According to other studies, ascorbic acid promoted corneal epithelial wound healing and the reconstruction of epithelial basement membranes thanks to the stimulation of epithelial stem cell formation.[21] In another clinical study, vitamin C supplementation was administered to 82 patients with infectious keratitis. Systemic vitamin C reduced the size of corneal opacity, especially in younger patients and those with hypopyon, with the intravenous vitamin C group (20 g/d) showing the best results compared to oral vitamin C (3 g/d).[23]

TRAM-34 is a selective inhibitor of calmodulin/calcium-activated K+ channels ($K_{Ca}3.1$). These channels mediate the downregulation of TGFβ-1-induced proliferation and differentiation of fibroblasts to myofibroblasts and may present a therapeutic target for the treatment and prevention of corneal fibrosis in *in vitro* studies.[2] The combination of TRAM-34 with ascorbic acid in an *in vivo* topical model of rabbit cornea showed an effective reduction of corneal fibrosis,[33] but this promising target has not been used yet in an infectious keratitis model. Likewise, curcuminoids present antiangiogenic properties in mice cornea models associated with the inhibition of MMP-9 and fibroblast growth factor-2.[72]

Amniotic membrane grafts are being used as another potential modality for the treatment of corneal ulcers associated with bacterial, viral, and fungal keratitis.[94] Rapid improvement of HSV-1-induced ulcerative stromal keratitis is noted after amniotic membrane transplantation on mice with corneal ulceration. This may be caused by reduced expression and activity of MMP-8 and 9, increased expression of TIMP-1, and sustained expression of TIMP-2.[43]

As already proven in sterile corneal inflammation, the plausible biological effects of all these molecules as adjuvants are interesting and may have a role in the management of infectious keratitis. The stability *in vivo* of these molecules and the select inhibition of defined MMPs involved in infectious keratitis may be the main limitations in clinical practice. Topical administration may have advantages, which includes more ease in the absorption and direct effect of the drug over the therapeutic target and the limitation of side effects; however, the controlled action of MMPs during human corneal infection must be proven in standard clinical experience. Unfortunately, currently, there are no high-quality randomized controlled trials in humans to guide clinicians in their proper use, despite their widespread use in clinical practice among corneal specialists.

5. Conclusion

Microbial keratitis is characterized by the production of collagenolytic substances with the consequent inflammation and corneal collagen degradation and also with the stimulation of physiological pathways that perpetuate the secretion of MMPs by epithelial, stromal, and inflammatory cells.

The control of excessive collagen destruction during microbial keratitis could be a goal to minimize corneal scarring after the infection, decrease the duration of the process, and avoid the consequences related to corneal stroma lysis, thinning, and perforation.

Some anticollagenase treatments, such as tetracyclines, reduce stromal destruction in clinical noninfectious ulcers. Although doxycycline treatment seems to produce the same effect in MMPs activity in an infectious keratitis context,

randomized and masked clinical trials are necessary to confirm its benefit.

The study of new substances, either systemic or topical, has shown potential in experimental and animal studies, but their use in human models could contribute to expanding the knowledge about the intricate relation between MMPs, corneal healing, the decrease in postinfectious corneal opacity, and the improvement of visual outcomes after corneal infections.

6. Method of literature search

A literature review was conducted using PubMed, Web of Science, and ScienceDirect platforms. The search was performed using the terms “metalloproteinases” or “MMPs” or “corneal metalloproteinases” or “collagenase” or “corneal collagenases” in combination with search words, such as “infectious keratitis,” “bacterial keratitis,” “viral keratitis,” “fungal keratitis,” and “acanthamoeba keratitis” for the first part of this review with the objective of describing the connections about corneal infectious and MMPs variations and activity.

The next search included “anticollagenolytic,” “anticollagenase treatment,” “collagenase,” and directed searches with the keywords “doxycycline,” “corticoid,” and “corticosteroids” combined with “keratitis,” “infectious keratitis,” and “corneal infection” with the objective to show the actual advances of anticollagenolytic therapy in corneal infection.

A total of 98 articles were found, and 22 articles were excluded because of not relevant information for this review. Relevant articles from the reference lists of identified articles were manually searched for additional inclusions. The respective references of articles were cross-matched to identify 35 more articles relevant for the review and finally included. The search was restricted to articles written in English. However, articles written in other languages with at least an English abstract were also considered if adequate information could be retrieved from the abstract. Articles without an English abstract were excluded. We included articles with the subject matter of interest for the description of the physiopathology of corneal MMPs in normal situations and during bacterial, viral, fungal, and acanthamoeba corneal infections.

We included all the retrieved articles, reviews, case reports, and clinical trials founded related to antimetalloproteinase treatment in infectious keratitis in clinical practice and experimental studies. Likewise, we excluded articles if the anticollagenase treatment was focused on different contexts other than infectious keratitis, such as chemical corneal burns. A few select articles published before 1990 are included for historical objectives, particularly to characterize corneal MMPs and biological pathways, including some articles describing antimetalloproteinase treatments in experimental studies in that decade.

7. Key references

Reference: 75 Nishida et al., 2021 by the description of extended in time corneal melting in infectious keratitis through corneal fibroblasts and inflammatory mediators. Moreover, the article provides an interesting resume about some of the actual antimetalloproteinase’s treatment.

Reference: 93 Sugioka et al., 2016 by clarify the molecular connection via of the activated keratocytes during corneal inflammation.

Reference: 48 Jamerson et al., 2020 by the complete information about the role of metalloproteinase 9 in ocular surface disorders, including infectious keratitis, and the useful drugs over metalloproteinase 9 activity.

Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Declaration of Competing Interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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